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EFFECTS OF PROBIOTICS ON PERFORMANCE OF CHICKENS UNDER NORMAL AND HEAT-STRESS CONDITIONS

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By

YASSIR WESAM ABDLZAHERA

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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By

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February 2016

Chairman : Liang Juan Boo, PhD
Institute : Tropical Agriculture

Poultry industry in most developing and newly developed countries in Asia is highly depending on importation of cheap feed ingredients at the sacrifice of quality. In view of the severe restriction or total ban on the use of antibiotics as growth promoters in livestock and poultry production, probiotic has been suggested as an alternative to antibiotics to promote productivity. Probiotic is a live microbial feed supplement which improves intestinal microbial balance and thus feed utilization and overall health of the host animal. Heat stress is one of the significant environmental challenges affecting poultry production all over the world, particularly in the tropics. Animals are considered to be under heat stressed if they have difficulty to achieve a balance between body heat production and body heat loss.

The overall objective of this study was to investigate the probiotic efficacy of mixture of two strains of *Lactobacillus*, namely *L. pentosus* ITA23 and *L. acidipiscis* ITA44 which were isolated from local Mulberry (*Morus alba*) silage on stress reduction of broiler chickens kept under hot environment. Two experiments were conducted to test the hypothesis that probiotic can effectively reduce stress and thus sustain productivity in chickens kept under hot environment.

The first experiment was conducted under ambient temperature in an open-sided poultry experimental house to examine the effect of the probiotic on performance, cecal bacterial population and relevant blood parameters in chickens. One hundred twenty day-old broiler chicks (Cobb500) were randomly allocated into two dietary treatments; commercial diet (control) and commercial diet supplemented with probiotic (1g /kg diet) in a 35-day feeding trial. Results showed that although there were no differences ($P>0.05$) in average daily weight gain (ADG), birds supplemented with probiotic had more efficient feed conversion ratio (FCR) compared to those without probiotic supplementation. The supplementation of the two strains of *Lactobacillus* tends to lower concentrations of serum total cholesterol, low density lipoprotein (LDL) and triglyceride, they were not significantly different with those in the control group.

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The second experiment was carried using 192 day-old chicks (Cobb500) to examine the effect of probiotic supplementation on broiler chickens under heat-stress condition .The birds were randomly allocated in equal number to two dietary groups similar to that in Experiment 1 and kept under ambient temperature for the first two weeks. On day-15, birds in each dietary group were randomly divided into two subgroups and transferred to temperature-control chambers; one subgroup from each dietary group to 25°C (low temperature) and the remaining half to 35°C (high temperature) for the next three weeks. The results showed that birds kept in the low temperature chamber had higher ADG than those in the high temperature groups, and supplementation of probiotic reduced the negative effect of high temperature on ADG of birds. Similarly, high temperature negatively affected FCR and probiotic supplementation improved FCR, with birds supplemented with probiotics in the low temperature group had the most efficient FCR while those without probiotic supplementation in the high temperature recorded the least efficient FCR.

Supplementation of the two strains of *Lactobacillus* did not alter total gut microbial population under both temperature conditions, but probiotic supplementation significantly ($P<0.05$) increased population of the beneficial bacteria (*Lactobacillus*, *Bifidobacter* and *Entrocucous*) in the cecum samples. However, probiotics only reduced population of pathogen (*Entrobacter* and *E. coli*) in the cecum samples of broilers kept under low temperature but not in those under high temperature.

Results of this study suggested that supplementation of the two strains of probiotic bacteria improved FCR. This could be because of the healthier gut environment as shown by the higher population of beneficial bacteria in cecum of birds fed probiotic as well as the higher antioxidant activity of these birds to cope with stress associated with high temperature.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PROBIOTIK PADA PRESTASI AYAM DALAM KEADAAN BIASA DAN TEKANAN HABA

Oleh

YASSIR WESAM ABDLZAHERA

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Industri ternakan ayam di kebanyakan negara membangun dan yang baru dibangunkan di Asia amat bergantung kepada pengimportan bahan-bahan makanan yang murah. Memandangkan penggunaan antibiotik sebagai pendorong pertumbuhan ternakan dan pengeluaran ayam telah dihadkan, probiotik telah dicadangkan sebagai alternatif kepada antibiotik bagi meningkatkan produktiviti. Probiotik adalah suplemen makanan berupa mikrob hidup yang boleh meningkatkan kesihatan dengan cara memperbaiki keseimbangan microbiota usus. Tekanan haba merupakan salah satu faktor alam sekitar yang menjejaskan pengeluaran ayam di seluruh dunia, terutamanya di kawasan tropika. Ayam dianggap berada di bawah tekanan haba jika dia menghadapi kesulitan untuk mencapai keseimbangan di antara penghasilan haba badan dan kehilangan haba badan.

Objektif keseluruhan kajian ini adalah untuk mengkaji keberkesanan dua jenis *Lactobacillus* iaitu *L. pentosus* ITA23 dan *L. acidipiscis* ITA44 yang telah diasingkan daripada silaj mulberi (*Morus alba*) bagi mengurangan tekanan haba ke atas ayam pendaging yang disimpan di bawah persekitaran yang panas. Dua eksperimen telah dijalankan untuk menguji hipotesis ini.

Eksperimen pertama telah dijalankan di bawah suhu ambien di dalam rumah ayam terbuka untuk mengkaji kesan probiotik pada prestasi, penduduk bakteria cecal dan parameter darah relevan pada ayam. Seratus dua puluh ekor anak ayam pendaging berumur sehari (Cobb500) telah dibahagikan secara rawak kepada dua kumpulan yang diberi makanan berlainan; diet komersial (kawalan) dan diet komersial yang ditambah dengan probiotik (1g / kg diet) selama 35 hari. Hasil kajian bagi purata pertambahan berat badan harian (ADG) tidak menenunjukan sebarang perbezaan (P> 0.05). Walaubagaimanapun, kumpulan ayam probiotik adalah lebih cekap dari segi nisbah penukaran makanan (FCR) berbanding dengan kumpulan kawalan. Suplemen kedua-dua jenin *Lactobacillus* cenderung untuk mengurangkan kepekatan jumlah kolesterol serum, lipoprotein ketumpatan rendah (LDL) dan trigliserida, keputusan ini adalah tidak ketara secara statistik berbanding dengan kumpulan kawalan.

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Eksperimen kedua dijalankan dengan menggunakan 192 anak ayam berumur sehari (Cobb500) untuk mengkaji kesan daripada suplemen probiotik pada ayam daging di bawah keadaan haba tekanan yang ditetapkan secara rawak kepada dua kumpulan makanan sama seperti Eksperimen 1 dan disimpan di bawah suhu ambien untuk dua minggu pertama. Pada hari ke-15, burung dalam setiap kumpulan makanan dibahagikan secara rawak kepada dua kumpulan kecil dan dipindahkan ke kamar kawalan suhu; satu kumpulan kecil dari setiap kumpulan pemakanan untuk 25°C (suhu rendah) dan separuh yang tinggal untuk 35°C (suhu tinggi) untuk tempoh tiga minggu seterusnya. Hasil kajian menunjukkan bahawa burung yang dibela pada suhu rendah mempunyai ADG lebih tinggi daripada mereka dalam kumpulan suhu tinggi, dan suplemen probiotik mengurangkan kesan negatif suhu yang tinggi pada ADG burung. FCR bagi kumpulan yang dibela pada suhu tinggi terjejas dan suplemen probiotik dapat m FCR. Ayam dalam kumpulan yang ditambah dengan probiotik dalam kumpulan suhu rendah mempunyai FCR paling baik manakala mereka yang tidak mempunyai makanan tambahan probiotik dalam suhu yang tinggi mencatatkan FCR kurang cekap.

Suplemen kedua-dua jenis *Lactobacillus* tidak mengubah jumlah mikrob usus di bawah kedua-dua keadaan suhu, tetapi suplemen probiotik meningkatkan populasi bakteria baik (*Lactobacillus, Bifidobacter dan Entrocucous*) dalam sampel cecum dengan ketara ($P < 0.05$). Walaubagaimanapun, hanya kumpulan probiotik sahaja menunkjukan penguarangan populasi patogen (*Entrobacter dan E. coli*) dalam sampel cecum ayam pendaging yang disimpan di bawah suhu rendah. Hasil kajian ini menunjukkan bahawa penambahan dua jenis bakteria probiotik memberi kesan positif kepada FCR. Ini mungkin kerana persekitaran usus yang lebih sihat seperti yang ditunjukkan oleh populasi bakteria bermanfaat yang lebih tinggi daripada dalam cecum burung diberi makan tidak probiotik.

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I certify that a Thesis Examination Committee has met on 19 February 2016 to conduct the final examination of Yassir Wesam Abdlzahera on his thesis entitled "Effects of Probiotics on Performance of Chickens under Normal and Heat-Stress Conditions" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

ADG	Average daily weight gain
ABTS	Radical scavenging capacity assay
AGP	Antibiotic growth promoters
ANOVA	Analysis of variance
bp	Base pair
BW	Body weight
BW	Body weight
BWG	Body weight gain
BWG	Body weight gain
CAT	Catalase activities
CFU	Colony forming unit
CT	Cycle threshold
EAAT3	Excitatory amino acid transporter
EU	European Union
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
FI	Feed intake
FRAP	Ferric reducing ability of plasma
g	Gram
GC	Gas chromatography
GPx,	Glutathione peroxidase
GSH	Glutathione
h	Hour
HDL	High density lipoprotein
HO1	Heme oxygenase 1
IACUC	Institutional Animal Care and Use Committee
IgG	Immunoglobulin
kg	Kilogram
LAB	Lactic acid bacteria
LCAD	long chain acyl CoA dehydrogenase

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LDL	Low density lipoprotein
MDA	Malondialdehyde
min	Minute
MJ	Mega joules
ml	Milliliter
MUFA	Monounsaturated fatty acids
PepT1	Oligopeptide transporter
pH	Potential of Hydrogen
PUFA	Polyunsaturated fatty acids
qPCR	Quantitative real-time PCR
RH	Relative humidity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Revolutions per minute
SAS	Statistical Analysis Software
SGLT1	Sodium -dependent glucose and galactose transporter
SGLT4	Sodium -independent glucose
SOD	Superoxide dismutase
TCA	Tricarboxylic Acid
TEAC	Trolox Equivalents Antioxidant Capacity
TG	Triglyceride
UFA	Unsaturated fatty acids
µl	Microlitre
VFA	Volatile fatty acids

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CHAPTER 1

INTRODUCTION

Among the agricultural sectors, poultry industry has played the leading role to provide animal protein (meat and egg) in many parts of the world. Chicken meat production has recorded much higher growth rate than any other type of meat during the past decade and its production has been on the increase in all continents with the highest increases in Asia and South America. Globally, the rate of increase in chicken meat production averaged 5.7% per year since 1990 (Daghir, 2008).

Poultry production is one of the agricultural activities which can effectively provide income and tackle the issue of underemployment in the rural communities, notably for the poor and marginalized farmers. Since nutrition plays a fundamental role in determining the health and performance of poultry and a correctly balanced diet is essential to avoid disease associated with a deficiency or toxicity of a particular nutrient, the biggest challenge of poultry production is the availability of quality feed at sustainable and stable price (Iyayi, 2008). Majority of the feed ingredient used for poultry production in many developing and newly developed countries in Asia are imported from different sources depending on availability and price, and often at the sacrifice of quality. Hence, Antibiotic Growth Promoters (AGP) had been widely added into feed to counter microbial infections and to promote growth in poultry and other livestock production. However, due to public health concerns the use of AGP in poultry (and other livestock) production is either been restricted or is banned in many industrialized countries, particularly in European Union (EU).

Probiotics, live microbial feed supplements which beneficially affecting the host animal by improving its intestinal microbial balance and exhibit a beneficial effect on the health of the host (Rolfe, 2000), have been suggested as alternative to AGP. Lactobacillus is one of the most common strains of the beneficial bacteria extensively studied and used as probiotics. Several studies have shown that the addition of probiotics to the diets of broilers led to improved performance (Jin et al., 1997; 1998), thus there are increasing interests to incorporate probiotics in the feed to minimize the use of antibiotics.

Probiotic bacteria can alter serum cholesterol and triglyceride levels (Lin et al., 1989; Taranto et al., 1998). Cholesterol is necessary for several functions in body because it acts as a presage to specific hormones and vitamins. It is also considered within the components of membranes and nerve cells. However, it is known that the high lipids content (such as triglycerides), or cholesterol in the blood is considered dangerous indicators for humans coronary heart disease development (Lim et al., 2004). Some evidences exist indicated that feed supplementation of *Lactobacillus* diminishes cholesterol and fatty acid compositions of broiler chickens (Kalavathy et al., 2006).

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In addition, probiotic bacteria could be considered as an option to reduce the adverse effects of heat stress in poultry industry. Heat stress is a significant environmental challenge affecting poultry production all over the world, particularly in the tropical regions, including Malaysia. Heat is produced by metabolic activities within the body, which include for maintenance, growth and egg production. Chronic heat stress has deleterious effects mainly through reducing feed consumption and increasing water consumption. High ambient temperatures coupled with high humidity can become more critical. Heat Stress not only causes suffering and death in the birds, also results in reduced or lost production that adversely affects the profitability of the enterprise.

Many strategies have been recommended to alleviate the negative effects of high environmental temperature on poultry performance; including manipulations of the amount and type of energy, protein, and other specific nutrients of the diet (Sahin et al., 2009). Supplementation of trace elements and dietary vitamins has also shown to contribute in alleviating the effect of heat stress. The use of probiotics may also be considered as a mean to overcome the adverse effects of heat stress.

Mechanisms by which probiotics improve feed conversion efficiency, enhancing growth of nonpathogenic facultative anaerobic and gram positive bacteria, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients are still poorly understood (Soccol et al., 2010), and their mode of actions are suggested to be multifaceted, also each probiotic may have specific functions affecting the host (Shine, 2012).

The hypothesis of this thesis was that the two strains of *Lactobacillus,* namely *L. pentosus* ITA23 and *L. acidipiscis* ITA44 which were isolated from Mulberry (*Morus alba*) silage by researchers from the Institute of Tropical Agriculture, Universiti Putra Malaysia could act as antibiotics to enhance growth performance of broiler chickens particularly under hot environment. Thus, the main objective of this thesis was to investigate the probiotic efficacy of the abovementioned two strains of *Lactobacillus on chicken* performance, cecal bacterial population and the relevant blood parameters.

The study consisted of two experiments. The aim of the first experiment was to study the probiotic efficacy *of L. pentosus* ITA23 and *L. acidipiscis* ITA44 on broiler chickens kept under ambient temperature in open-sided poultry house. The effects of the above *Lactobacillus* on feed intake, growth performance, changes in the bacterial population in the cecum and serum cholesterol were studied. Based on the results of the first experiment, a follow-up experiment was conducted to test the hypothesis that the supplementation of probiotic will alleviate the negative effects of heat stress on broilers, and also the positive effects of probiotics are more pronounced in broiler birds kept under high environment temperature (35°C) condition. Feed intake, growth performance, changes in bacterial population of cecum, antioxidant capacity of liver, volatile fatty acids of cecal contents, expression of nutrient absorption genes

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and fatty acid profile of the breast tissue were used as the basis of the effect of probiotic on heat stressed birds.

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